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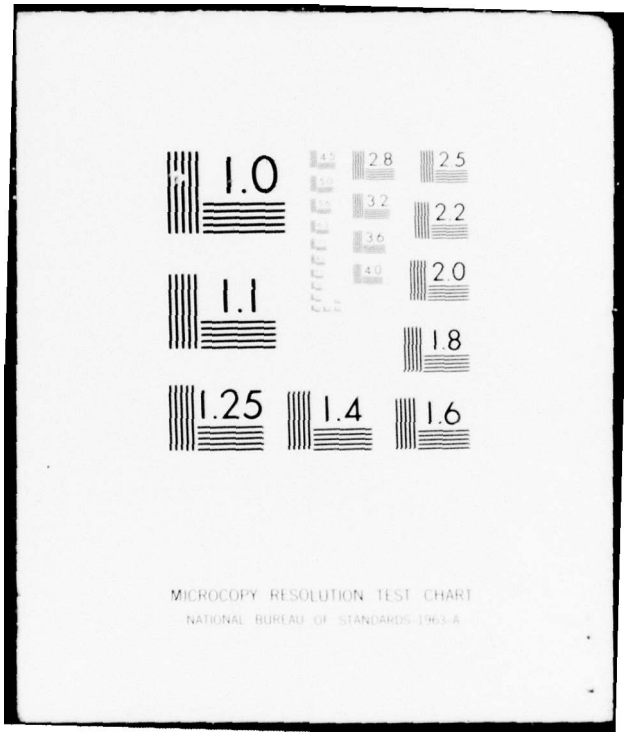
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by

Jacek Dutkiewicz and Czeslaw Kwapiszewski

EDITED TRANSLATION

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A NEW APPARATUS TO STUDY THE MICROBIOLOGICAL CONTAMINATION OF AIR

(NOWY APARAT DO BADANIA MIKROBIOLOGICZNEGO ZANIECZYSZCZENIA POWIETRZA)

Jacek Dutkiewicz, Czeslaw Kwapiszewski

Institute of Labor Medicine and Rural Hygiene
Lublin

Description, principles of action and potential application are given for a new apparatus which takes simultaneously two microbiological samples of air onto Agar cultures. One of these samples gives data to calculate the total number of microorganisms in air, while the other enables one to determine the size of respirable fraction of microorganism aerosol. This fraction is separated out by means of a selective arrangement consisting of a number of tubes and of regulated shield settlers. An accumulator as a power source and parameters of electrical parts of the apparatus enable to carry out a broad range of determinations under any conditions.

Among various methods to measure the microbiological contaminants in air /11, 13, 16/, the most often used currently is the impact or inertial method. Basically, this method draws, by means of a pump, a determined volume of investigated air through a narrow slit or hole in hermetic chamber onto a plate with a constant Agar culture. As a result of impact of the fast flowing stream of air with the culture, small particles of aerosol

fall out of the stream and adhere to the surface of Agar. After the incubation of cultures in thermostat and counting the number of grown colonies of microorganisms - or, more correctly, of microorganism particles - the number is calculated of "viable particles" in 1 m^3 of air according to a simple formula /13/, and a qualitative composition of microflora is determined.

Among the instruments constructed on the basis of the impact method one can distinguish those which take only one sample of air each time, and those which take simultaneously several samples of air and, at the same time, make separation of aerosol particles according to size. The first group of instruments comprises a number of slit apparatuses, the most known of which is an apparatus designed by Boudrillon and collaborators /2, 3/ and produced in various versions by the firm Casell in London. In this apparatus, the air is drawn onto the plate with culture in hermetical chamber through a narrow slit in metal cylinder inserted inside the chamber. The plate is placed on a rotating disc to ensure the uniform distribution of deposits on culture. A similar constructional solution is found in the apparatus of Krotov /12/ and other related instruments /13/, in which the slit is located in a Plexiglas or glass cap placed onto the top part of arrangement containing the plate with culture.

These instruments do not allow to separate and determine quantitatively the fine respirable fraction of microorganism aerosol, comprising fine particles ($< 3\ \mu m$) able to penetrate the pulmonary alveola /10, 11/. Such determinations, which are

extremely important from medical and zoohygienic viewpoint, are possible, however, by means of instruments of the second group, in which the air stream is directed in turn through the slits or openings with smaller and smaller diameters onto plates with cultures placed one under the other. This is the so-called cascade method of sampling the air, applied for the first time by May /14/ in a cascade instrument to study dust deposition, AND being able to separate particle fractions by size from the largest to the smallest one. This method was applied in a "TDL" instrument consisting of four consecutively connected Bourdillon apparatuses each having a smaller size of slit than the preceding one, /16/. Undoubtedly, the best known cascade apparatus for microbiological purposes is the Andersen apparatus /1/, produced commercially. In this apparatus, air samples are taken onto 6 plates with culture, placed consecutively under 6 metallic sieves with 400 calibrated holes, smaller and smaller in each next sieve. In this instrument, the respirable fraction of microorganism aerosol is isolated on cultures placed under sieves with holes of the smallest diameter, i.e., in segments 5 and 6.

All the above-described instruments have many good points along, however, with some shortcomings, which are particularly noticeable when making studies under field conditions. The majority of them are powered with 220 volts AC, so that they cannot be used if such a network is not available /11/. The construction of certain instruments (Krotov, Andersen) requires that very precise volumes of Agar are deposited on plates, so that

the preparation of cultures is complicated. The need is felt also of an apparatus which would allow to perform accurate and full determinations of air samples containing large number of microorganisms. Such conditions exist, for instance, in places contaminated with organic dusts (barns and stables, grain storage facilities, breweries, etc.). Microbiological studies of air in such environments are of considerable medical importance, since some microorganisms appearing in dusts represent a potential factor of professional health hazard mainly because of allergenic properties /7, 8, 15/. Such studies have been in progress for a number of years at the Institute of Labor Medicine and Rural Hygiene in Lublin /5, 6/, using a modified Bourdillon apparatus /4/. The modification, by eliminating the above discussed shortcomings allows to obtain good results under field conditions. It does not allow, however, to isolate the respirable fraction of microorganism aerosol. This fraction could be isolated by application of the Andersen apparatus and other instruments based on the cascade air sampling. However, the use of these instruments in microbiological studies of air contaminated with organic dusts presents many difficulties, which do not allow to perform accurate determinations of the number of microorganisms in air and of the size of respirable fraction of microorganism aerosol. This arises mostly because during the cascade flow of air through a number of narrow slits a considerable part of dust is retained on the walls of apparatus. Moreover, a certain portion of fine aerosol particles settles down on plates placed under the larger holes in the first

segments of apparatus, changing the experimental results.

In order to eliminate the shortcomings and inconveniences of instruments used currently, the Institute of Labor Medicine and Rural Hygiene designed an apparatus for microbiological studies of air contamination which would allow to take samples under all field conditions and, at the same time, would permit to determine the total number of microorganisms in the unit volume of air, the size of respirable fraction of microorganism aerosol, and composition of the microflora of the air. After construction of the prototype and its successful testing under field conditions, the description of apparatus was submitted to the Polish Patent Office, applying for patent under the number P-166 753.

Design features of the new apparatus

A new method of taking microbiological samples of air for determination of the respirable fraction of microorganism aerosol was applied in order to eliminate losses connected with the cascade sampling of air. In this method, two samples of air are taken simultaneously through two intakes onto plates with Agar cultures placed in separate hermetical chambers. One of these plates, onto which the stream of air comes directly through the length of nozzle, collects the total amount of microorganism aerosol present in the unit volume of air sampled. The stream of air directed onto the second plate passes through a selective arrangem[illegible]ttached to the second nozzle. This arrangement captures

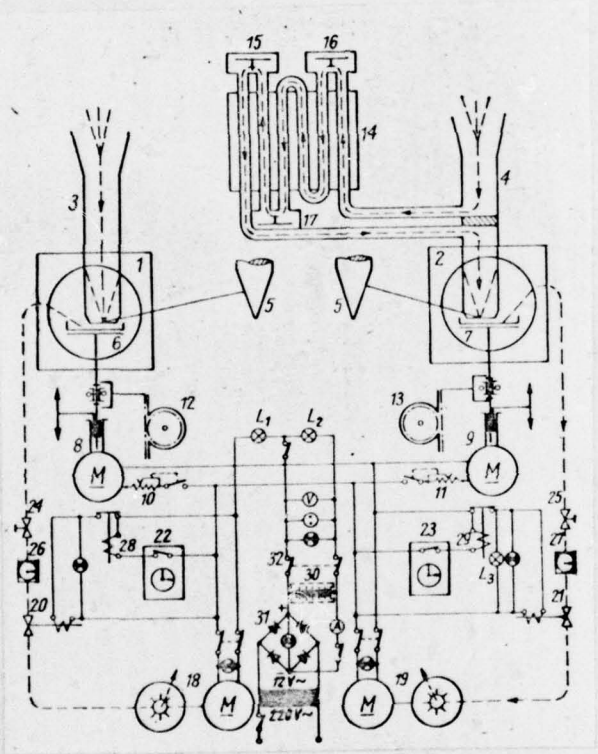

Figure 1. Diagram of the apparatus. 1, 2 - hermetic chambers; 3, 4 - intake nozzles; 5 - conical tip; 6, 7 - metal plates; 8, 9 - electrical motors; 10, 11 - resistance arrangements; 12, 13 - lifting mechanisms; 14 - selective arrangement; 15, 16, 17 - shield depositors; 18, 19 - electrical motors; 20, 21 - electromagnetic valves; 22, 23 - time turnoffs; 24, 25 - regulating valves; 26, 27 - flowmeters; 28, 29 - contacts; 30 - accumulator; 31 - rectifier; 32 - main switch.

coarse-grained particles, so that the plate is reached only by the fine respirable fraction of microorganism aerosol. The selective arrangement mentioned, i.e. artificial breathing filter, consists of a system of tubes and regulated shield depositors coated with a sticky substance. In order to be able to sample air under any condition the apparatus is equipped with an accumulator which provides direct current to all the electrical devices mounted permanently: intake pumps, motors rotating the dishes with plates, and resistance arrangements regulating smoothly these rotations in a broad range, electromagnetic valves and time switches joined into an assembly enabling the automatic sampling of precisely measured quantities of air, flowmeters, and a number of other facilities assuring an efficient functioning of the apparatus.

Construction of the apparatus

The diagram of the apparatus is shown in Figure 1. Two hermetical chambers 1, 2 are placed on the body of the apparatus. In the upper part of each chamber there are intake (sucking in) nozzles of the same diameter 3, 4 with conical tip 5 and a slit 30 mm long and 0.3 mm wide. Inside of each chamber there are metallic PLATES 6, 7 onto which Petri dishes with Agar culture are placed for collection of samples. Electric motors 8, 9 impart rotating motion to these PLATES. Resistance devices 10, 11 ensure smooth regulation of the rotation velocity of PLATES in the range 10 to 60 rpm. The distance of plates with dishes from the nozzle outlet is regulated by a suitable lifting

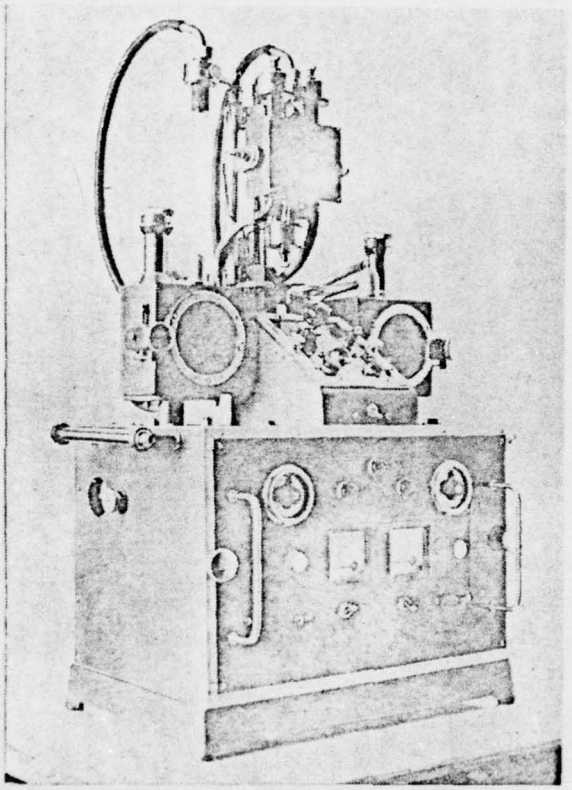

Figure 2. View of the assembled apparatus.

mechanisms 12, 13 , and the correct adjustment of distance between the nozzle outlet and the surface of Agar is done with the aid of A SCALE (not shown on Figure). The insides of the chambers are lighted with light points L_1, L_2. A selective arran gement 14 in rectangular case is attached to the intake nozzle 4 of chamber 2.

It consists of six glass tubes of diameter 10 mm and three regulated shield depositors 15, 16, 17 coated with a gluey substance on which the coarse-grained fraction of the dust becomes deposited. The selective unit has its own lighting L_3 which enables to follow the flow of air containing dust.

The flow of air, shown in the figure by the broken line, is assured by the air pumps driven by electric motors 18, 19 of power 120 W and 7000 rpm. Electromagnetic valves 20, 21 and time switches 22, 23 serve to turn on and off automatically the flow of air onto the plates. The flow of air through the chambers is regulated by means of regulating valves 24, 25, flowmeters 26, 27 and contacts 28, 29. The lower part of the apparatus contains an accumulator 42 Ah 30 and a rectifier 31. The complete turning off of the apparatus is possible by means of a built-in main switch 32. All these enumerated electrical parts are on the outer case of the apparatus. On the front part of this case there is a panel with a voltmeter, amperometer, pump switches, regulating knobs, signal lights, and clock faces of time switches. The appearance of the apparatus assembled and after partial dismantling is shown in Figures 2 and 3.

Functioning of the apparatus

The taking of air samples by means of the apparatus proceeds in the following way. After turning on of the MAIN switch and lighting the chambers, which is necessary for the proper placing of the plates with culture under the outlets of

the nozzles, the chambers are opened and the uncovered dishes with a suitable Agar culture are put in. Then the plates with culture are set, by means of mechanisms of vertical regulation and a graduated scale contained in the chambers, in such a way that the surface of Agar is at the distance of 1-2 mm from the conical TIP of the intake nozzles. After closing the chambers, the motors moving the plates with dishes are turned on, and the desired rotation velocity of these plates is set by means of knobs connected with the resistance unit. Next, the time switches based on clock mechanisms are set for an appropriate time of exposure and then the intake pumps are turned on and the required flow of air (the most useful 20 to 25 l/min) is adjusted by means of regulating valves and flowmeters. Then, by turning on the time switches, the electromagnetic valves begin operating, starting the flow of air through the chambers for a period of time set previously on switches, enabling in this time to collect samples through the intake nozzles on both the plates. After the preset time passes, the electromagnetic valves automatically close the flow of air through the chambers. After taking the samples, the pumps and motors WHICH ROTATE plates with dishes are switched off, the dishes are lowered, the chambers are opened and the dishes are taken out and the AGAR culture covered. After these operations, the lights in chambers are turned off and the main switch is closed.

The plates onto which the samples of air were taken are put into an incubator for the required time of incubation, most often 24-48 hours at the temperature 37°C, and the number of colonies of microorganisms on cultures in both chambers is calculated. On this general basis, the number of microorganism particles in 1 m^3 of air is determined, as is the value of respirable fraction, or the value expressing the percentage of microorganisms which could penetrate the pulmonary alveola, Then, identification of colonies is made and the qualitative composition of the air microflora is established both for the total sample and for the respirable fraction.

When using the described apparatus, it is possible to employ various substrates and various times and temperatures of incubation, depending on the type of investigation and the kind of microorganisms sought.

Results of the field tests of the apparatus

Using the new apparatus we collected microbiological samples of air in grain storage facilities, and on farms where animals (pigs) were raised. The number of bacteria and other microorganisms was determined on the basis of air samples taken onto Agar cultures with addition of 5% of ram's blood - and the number of molds on the basis of samples taken onto the Sabouraud culture. For comparison purposes, parallel samples onto analogous cultures were taken also using the previously mentioned modified Bourdillon apparatus. In the tests in grain stores, the total

number of bacteria in the air, determined on the basis of samples in chamber 1, was 1608.6 thousands/m^3, while the number of bacteria composing the respirable fraction, determined on the basis of samples in chamber 2, was 887.0 thousands/m^3, i.e., 55.1% of the total amount. With respect to molds, analogous values were correspondingly 24.0 thousands/m^3 and 9.6 thousands/m^3 (40.0%). Difference in the number of bacteria colonies on cultures exposed simultaneously in chamber 1 and chamber 2 (FIGURE 4a AND 4b). In comparison with the modified Bourdillon apparatus, the effectiveness of the new apparatus proved to be higher by 19.5% on the average.

In the animal-breeding farms, the total number of bacteria in the air was 7748.2 thousands/m^3, and the number IN respirable fraction - 4477.9 thousands/m^3 (57.8%). Such a high amount of bacteria was impossible to determine accurately by the Bourdillon apparatus. The total number of molds was only 3.3 thousands/m^3, while no molds were found on cultures in chamber 2.

In order to check the effectiveness of the action of the selective unit with depositors set in the medium position, as in taking samples onto the cultures, samples of air were taken also onto basic glasses placed on non-moving plates directly under outlets of nozzles in chambers 1 and 2 , also in grain storage buildings and in animal-breeding farms. The glasses were coated previously with celophane soaked in the mixture of gelatin and glycerin (dissolved hot in the proportions: gelatin - 16 g, distilled water - 96 ml, glycerin - 112 g, and phenol - 2 g),

as is practiced normally when taking air samples by means of the cascade apparatus of May /14/. After the exposure, strips of celophane with traces of dust were cut out and MOUNTED permanently in the mixture of gelatin and glycerin. Subsequently, diameter of particles was measured in a phase-contrast microscope, and the dispersion analysis of aerosol particles was carried out, determining the geometrical mean of their distribution /16/. The results of this analysis for air samples taken in the grain storage buildings are presented on graphs illustrating the size distribution of aerosol particles in both chambers of the apparatus (Figure 5). It follows from these graphs that the geometrical mean of the distribution of particles in chamber 2 (2.1 μm) was less than a half in comparison with chamber 1 (4.6 μm). Similar results were obtained as a result of dispersion analysis of the particles of aerosol in animal-breeding farms, being respectively 2.5 μm and 4.8 μm.

The obtained results demonstrated the effectiveness of the new apparatus under field conditions, and confirmed a large degree of HAZARD to which people are exposed when staying in environment contaminated with organic dusts. This danger arises from the high level of microorganism contaminants in air and from high values of respirable fractions of the microorganism aerosol.

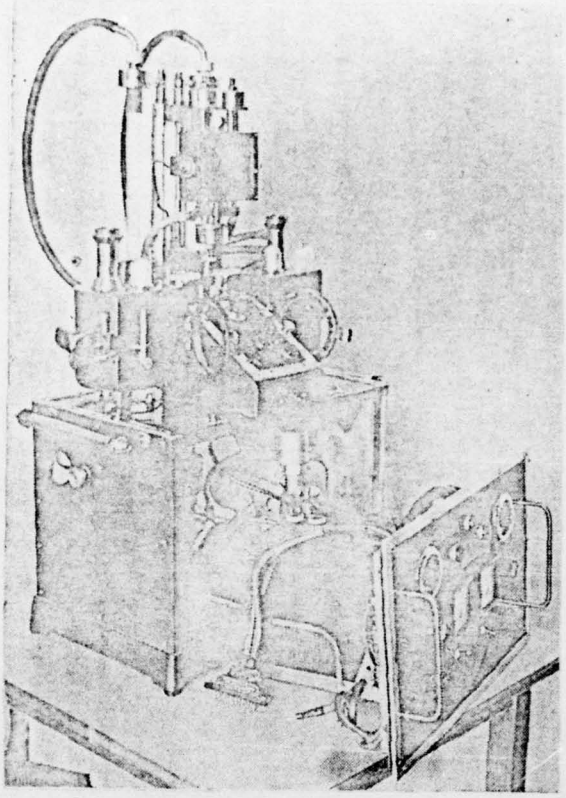

Figure 3. View of the apparatus after partial DISMANTLING

Characteristics of the apparatus

The method of selective study of microbiological contaminants of air, adopted in this apparatus, has the main advantage that it allows to collect simultaneously two samples, one of which contains all the particles of microorganism aerosol and forms the basis for determination of the total amount of microorganisms in air, and the other, on the other hand, contains only the respirable fraction of this aerosol and allows to determine the number of microorganisms capable to penetrate the pulmonary alveola. Thus this method makes it possible to perform

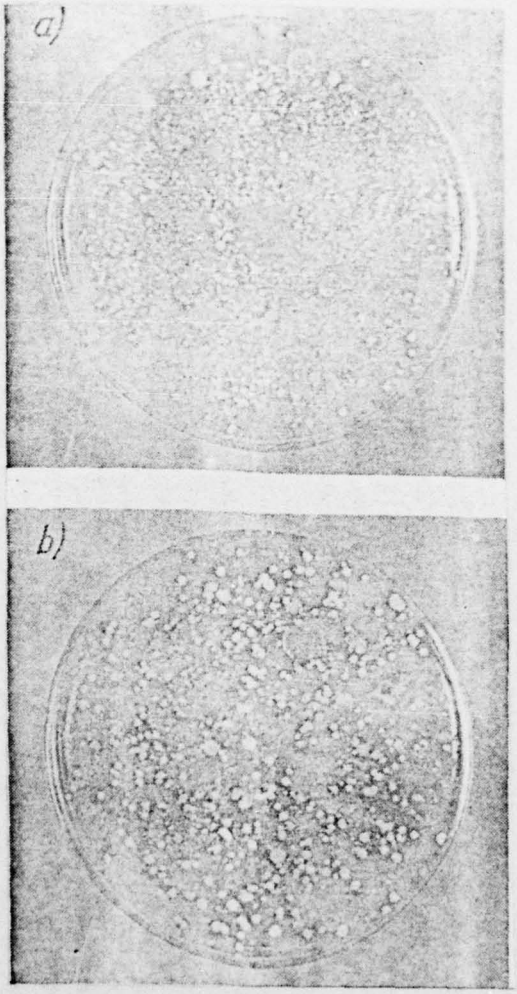

Figure 4. Microbiological samples of air, taken in grain storage buildings onto plates with the Agar culture with blood by means of the new apparatus; a) - sample taken in chamber 1 and containing totmal amount of bacterial aerosol; b) - sample taken in chamber 2 and containing respirable fraction of the bacterial aerosol.

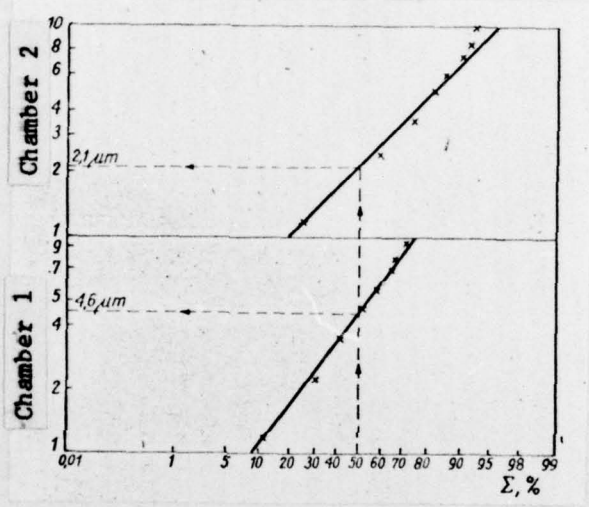

Figure 5. Size distribution of aerosol particles in chamber 1 and 2 (samples from grain storage buildings). The graph was made on logarithmic scale. The ordinate shows the size of aerosol particles in µm, and the abscissa - the cumulative percentage of the number of particles. Values of the geometrical means of particle distribution for both chambers are written over broken lines drawn for determination of these values.

the complete evaluation of the degree of HAZARD caused by the microflora of air, which cannot be done by application of apparatuses taking samples on one plate only (apparatuses of Bourdillon, Krotov, and others). Application of the method of simultaneous taking of samples eliminates large losses unavoidable when employing the cascade sampling, and enables to perform the precise and at the same time simple determination of the number of microorganisms in the unit volume of air, and also of the size of respirable fraction, which, for instance, is impossible with the Andersen apparatus. The elimination of losses is also AIDED BY THE application of conical intake nozzles instead of slits which appear in the Bourdillon apparatus. Construction of the selective unit allows, through regulation of separation distance of shield depositors, to isolate a fine-grained fraction of defined dispersion, if this is necessary in investigations.

The electrical system of the apparatus allows for a large qualitative and quantitative range of determinations of the microbiological contamination of air. The large output of pumps in combination with fast rotations of plates with dishes and with setting of short periods of exposure (1-10 seconds) on the clocks of time switches, enables to make precise determinations at particularly large numbers of microorganisms in air, i.e., in an environment contaminated with organic dusts. Such determinations are not possible with the currently known apparatuses, because of the already-discussed losses, and because they take for testing only large samples of air so that the number of colonies on plates

cannot be counted under conditions of very large contamination of air.

The own source of energy in the form of an accumulator, the pumps built-in permanently, and convenient handles for carrying the apparatus allow to take samples in any location. The inside lighting of the chambers allows to set with precision the surface of culture with respect to nozzle, and, in general, to take samples under MARGINAL lighting conditions . It should be also noted that the regulation of the distance of culture from the nozzle eliminates the necessity of placing on dishes of an accurately measured volume of Agar, which is required, for instance, in the apparatuses of Krotov and Andersen, and which creates additional difficulties in preparation of cultures.

In addition to the basic purpose, which is determination of microbiological contaminants of air, the discussed apparatus CAN be adapted also to perform determination of the contaminants of air of different character, such as chemical and mechanical one. This can be achieved by a suitable replacement of inlet nozzles, which can be DONE in very simple way.

The only disadvantage found in this prototype is a considerable own weight which, however, was not prohibitive in frequent successful use of the apparatus in the field, where it was carried by two persons. This disadvantage IS DUE TO THE HEAVY weight of the accumulator used and could be easily alleviated, in the case of larger production of the apparatus, by application of a much lighter accumulator of another type.

There is also further possibility of the reduction of weight through the replacement of some metal elements by plastics. The handling of the apparatus can be facilitated further by application of some special hand pushcart.

Conclusions

1. The new apparatus allows to determine accurately the total number of microorganism particles in a unit volume of air, and to determine the size of respirable fraction of microorganism aerosol which could penetrate pulmonary alveola.
2. The apparatus permits to perform under various conditions the evaluation of microbiological health hazards, even in the case of air very strongly contaminated with microorganisms such as, for instance, in an environment contaminated with organic dusts.
3. There is possibility of an adaptation of apparatus to perform determinations of the contamination of air with impurities of other character.
4. The described apparatus could find broad utilization in studies connected with protection of the natural environment of man, as well as in studies in the fields of medicine, agro-technology and zoo-technology.

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AFIT/LD		1

ORGANIZATION		MICROFICHE
E053	AF/INAKA	1
E017	AF/ RDXTR-W	1
E404	AEDC	1
E408	AFWL	1
E410	ADTC	1
E413	ESD	2
	FTD	
	CCN	1
	ETID	3
	NIA/PHS	1
	NICD	5